ELSEVIER

European Journal of Pharmacology 308 (1996) 87–96

ejp

Comparison of vasopressin and oxytocin receptors in the rat uterus and vascular tissue

Abdelmajid Anouar [a], Marie-Stéphanie Clerget [a], Thierry Durroux [b], Claude Barberis [b], Guy Germain [a,*]

[a] *Laboratoire de Neurobiologie des Fonctions Végétatives, Inra, 78352 Jouy en Josas Cedex, France*

[b] *Inserm U 401, Centre de Pharmacologie Endocrinologie, rue de la Cardonille, 34094 Montpellier, Cedex 05, France*

Received 9 November 1995; revised 19 March 1996; accepted 2 April 1996

Abstract

Several studies indicate that oxytocin and vasopressin receptors in the human uterus are heterogeneous. We have investigated whether oxytocin and vasopressin bind to separate receptors in the day 21 and day 22 pregnant rat uterus and whether uterine vasopressin receptors are the same as the vascular V_{1A} subtype. In isolated organ bath experiments we showed that the potency of $d(CH_2)_5$[Tyr(Me)^2]vasopressin to inhibit vasopressin contraction in rat aorta was different from that in the day 21 pregnant uterus. Saturation curves of [^{3}H]vasopressin in membranes from cultured aortic myocytes and pregnant uterus were linear and yielded the same 1 nM K_d values. However, the potency of $d(CH_2)_5$[Tyr(Me)^2]vasopressin and of [Thr^4,Gly^7]oxytocin at antagonizing [^{3}H]vasopressin confirmed the differences between the vascular smooth muscle and uterine vasopressin receptor. The peptides had respectively higher and lower affinity for aortic cell sites than for uterine sites. It was more difficult to distinguish pharmacological differences for oxytocin and vasopressin receptors in the uterus. On day 22, the high affinity of [Thr^4,Gly^7]oxytocin and oxytocin for both [^{3}H]oxytocin and [^{3}H]vasopressin binding sites was consistent with the notion that the uterus expresses essentially oxytocin receptors at this stage of gestation. However, oxytocin, vasopressin and three analogs showed a different potency for inhibiting [^{3}H]oxytocin and [^{3}H]vasopressin binding on day 21 versus day 22 of gestation. We conclude that in the rat uterus vasopressin binds to a receptor that is different from the vascular V_{1A} subtype. Also, the binding sites for [^{3}H]vasopressin and [^{3}H]oxytocin on day 21 uterus membranes do not resemble the classical oxytocin receptor as described in the literature, suggesting that on day 21 vasopressin and oxytocin bind in the uterus to a receptor that might be different from those currently characterized.

Keywords: Vasopressin receptor; Oxytocin receptor; Uterus; Pregnancy; (Rat)

1. Introduction

Just before parturition, the uterus changes from a hypocontractile state to a hypercontractile state, allowing the expulsion of the fetus. The neurohypophysial nonapeptide oxytocin is probably involved in this expression of uterine activity. Uterine sensitivity to oxytocin (Caldeyro-Barcia and Sereno, 1959) and oxytocin binding site number (Alexandrova and Soloff, 1980a,b; Maggi et al., 1990; Tence et al., 1990) increase at the time of parturition. An inhibition of uterine contractions during labor can be obtained with specific oxytocin receptor antagonists (Akerlund et al., 1987). However, another neurohypophysial nonapeptide, vasopressin, which differs from oxytocin by only two amino acids, also has significant uterotonic activity (Maggi et al., 1988; Chan et al., 1990). To date, several authors have reported the presence of separate oxytocin receptors and V_{1A}-like vasopressin receptors in the human and rabbit uterus (Guillon et al., 1987; Ivanisevic et al., 1989; Maggi et al., 1988, 1990; Tence et al., 1990). The uterine vasopressin receptor seems to be expressed essentially in the pregnant uterus before the onset of parturition. However, whether these vasopressin receptors really are of the same type as the vascular V_{1A} receptor was questioned by Chan et al. (1990), who raised the possibility of the existence of another vasopressin receptor type in the rat uterus. To answer these questions, we examined the phar-

* Corresponding author. Laboratoire de Neurobiologie des Fonctions Végétatives, Inra, Centre de Recherches de Jouy, Domaine de Vilvert, 78352 Jouy en Josas Cedex, France. Tel.: (33) 1 34 65 25 01; fax: (33) 1 34 65 25 05; e-mail: germain@jouy.inra.fr.

PII S0014-2999(96)00258-0

macological and binding properties of vasopressin receptors expressed in the rat uterus and compared them with those of the vascular V_{1A} receptor type and those of the uterine oxytocin receptor type.

2. Materials and methods

2.1. Animals

Pregnant (day 1 = sperm positive) Wistar rats were purchased from C.E.R.J., France, and caged in groups of four at 22°C under a 14 h light/10 h dark cycle. They were killed at 9:00 a.m. on either day 21 (non-parturient, group D21) or day 22 (parturient, group D22) of gestation.

2.2. Chemicals

Vasopressin, oxytocin and [Thr^4,Gly^7]oxytocin were purchased from Neosystem (Strasbourg, France). d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin: [1-(β-mercapto-β,β-cyclopentamethylene propionyl)-2-*O*-methyltyrosine-8-arginine]vasopressin and d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin: [1-(β-mercapto-β,β-cyclopentamethylene propionyl)-2-*O*-methyltyrosine-8-ornithine]vasotocin were a gift from M. Manning (Toledo, USA). [^{3}H]vasopressin (64.8 Ci/mmol) and [^{3}H]oxytocin (36.6 Ci/mmol) were purchased from N.E.N. DuPont de Nemours, France. All the other chemicals were of the highest grade available from Sigma, Merck and Prolabo.

2.3. Concentration-response curves of isolated uterus and aorta in organ baths.

Rats were killed by cervical dislocation at 9:00 a.m. after light anesthesia with diethyl ether. Uterine horns were dissected out and the fetuses discarded. Circularly oriented strips (≈ 25 mg/strip) were isolated from each horn and mounted in organ baths (10 ml volume) to be processed in groups of eight. Circular strips from the same animal were processed in parallel in a given experiment and equilibrated in a gassed (95% O_2-5% CO_2) physiological salt solution at 32°C composed of (mM): glucose, 2.8; KCl, 6.2; NaCl, 144; $CaCl_2$, 2.5; $MgSO_4$, 0.5; NaH_2PO_4, 1; $NaHCO_3$, 30; pH 7.4.

Thoracic aorta were taken from D21-pregnant rats. They were dissected and 8 rings (≈ 10 mg/ ring) were cut out, placed in an organ bath and equilibrated in a gassed solution (95% O_2-5% CO_2) at 32°C containing (mM): glucose, 11; KCl, 4.7; NaCl, 118; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; pH 7.4. The responses of rings were recorded in the presence of indomethacin, N^{ω}-nitro-L-arginine, phentolamine, propranolol and atropine, all at a concentration of 10 μM. The preparations were allowed to equilibrate for 1–2 h under a resting tension of 0.5 g for uterus or 1 g for aorta.

The contractile effects in aortic and myometrial strip preparations were recorded by computerized calculation of the integral under the tension-time curve for 2 min after the antagonist was added, as described by El Alj et al. (1993). Responses to cumulative doses of vasopressin were recorded in the absence (control) or in the presence (preincubation of 20 min) of two antagonists, d(CH$_2$)$_5$-[Tyr(Me)^2]vasopressin and d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin. Curves were fitted (GraphPad Software, San Diego, USA) using the logistic equation $E_A = (E_{max} \times C^n/[C^n + EC_{50}^n]) + E_0$, where E_A is the response; C, the agonist concentration; E_0, the baseline activity; E_{max}, the maximal response; EC_{50}, the agonist concentration resulting in a response corresponding to $E_{max}/2$ and n, the slope (pseudo-Hill coefficient). We consistently found that calculated values of n were without any significant variation within samples from the various tissue groups (results not given). $pK_B = -\log K_B = \log (A'/A - 1) - \log B$ was calculated according to Arunlakshana and Schild (1959). K_B is the dissociation constant of the antagonist and the agonist dose-ratio A'/A was determined only in the presence (1 nM or 100 nM) and absence of the antagonist B. Hence, the slope of the Schild plot was not determined. Quantitative values were compared using Student's unpaired t-test. Significance was set at $P < 0.05$.

2.4. Vascular smooth muscle cell culture

Aortic smooth muscle cells were obtained by the explantation method of Ito et al. (1994). Confluent monolayers with a cell density of 150000 cells/Petri dish were obtained after 3 weeks. Cultured cells were passaged by harvesting with trypsin (0.05%) + EDTA (0.02%) and seeded in Dulbecco's minimum essential medium (Gibco) containing 10% fetal calf serum. The medium was changed every 3 days. After five passages the cells were used for preparation of membrane fractions. Prior to their use, cells were incubated for 72 h in Dulbecco's minimum essential medium without fetal calf serum and phenol red but with ascorbic acid (0.2 mM), transferrin (5 μg/ml) and glutamine (2 mM).

2.5. Membrane preparations

2.5.1. Pregnant rat uterus

Membranes were prepared as described by Elands et al. (1988). They were purified by sucrose gradient centrifugation at 100000 × g for 2 h. The fraction sedimenting between 10% sucrose-1 mM EDTA and 35% sucrose-1 mM EDTA was isolated and resuspended in 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$ and centrifuged at 100000 × g for 20 min. The pellet was resuspended in the same buffer and frozen at a protein concentration of 1 mg/ml at −80°C until used.

2.5.2. Aorta myocytes

Confluent cultured cells were recovered by scraping off the Petri dish into 15 mM Tris-HCl, pH 7.4, 0.3 mM EDTA, 2 mM $MgCl_2$. Cells were then homogenized with a polytron and centrifuged at 50 000 × *g* for 20 min. The pellet was washed and centrifuged twice at 50 000 × *g* for 20 min in 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$. The pellet was resuspended in the same buffer at a protein concentration of 5 mg/ml and frozen at −80°C until used.

Protein concentration was estimated according to Bradford (1976).

2.6. Binding assays

Experiments were performed in duplicate on at least two different pools of membranes. For each experiment, the linearity of specific binding with protein concentrations was verified. Saturation analysis with [^{3}H]vasopressin was performed in membranes from aorta myocytes (60 μg) and D22 uterus (30 μg). Saturation analysis was also performed with [^{3}H]oxytocin in D22 uterus (30 μg). The assay used various concentrations (from 0.05 to 25 nM) of [^{3}H]oxytocin or [^{3}H]vasopressin. Non-specific binding was determined in the presence of the corresponding unlabelled peptide (10 μM). Binding reactions were carried out at 25°C for 90 min in a total volume of 200 μl of 100 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$ and 2 mg/ml BSA. Incubation was started by the addition of membranes and terminated by filtration (Whatman GF/C filter). The saturation parameters were estimated by Scatchard analysis using Ebda-Ligand software (Elsevier-Biosoft, Cambridge, UK). Self- and cross-displacements between [^{3}H]oxytocin or [^{3}H]vasopressin (0.5 nM) and various analogs (1 pM to 1 μM) with differing affinities for neurohypophysial hormone receptors (oxytocin, vasopressin, [Thr^4,Gly^7]oxytocin, d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin and d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin) were performed in the same incubation buffer as described above. Non-specific binding was determined in the presence of the corresponding unlabelled ligand (10 μM). Membranes from aorta, D21 uterus and D22 uterus were used at concentrations of 40 μg, 40 μg and 30 μg respectively. Ebda-Ligand software was used for a simultaneous analysis of families of self- and cross-competition curves and calculation of K_i's for competing ligands. Selection of the best model involving one or two independent classes of sites and significance of differences for parameter values were based on a *F*-test according to Munson and Rodbard (1980). K_i values were

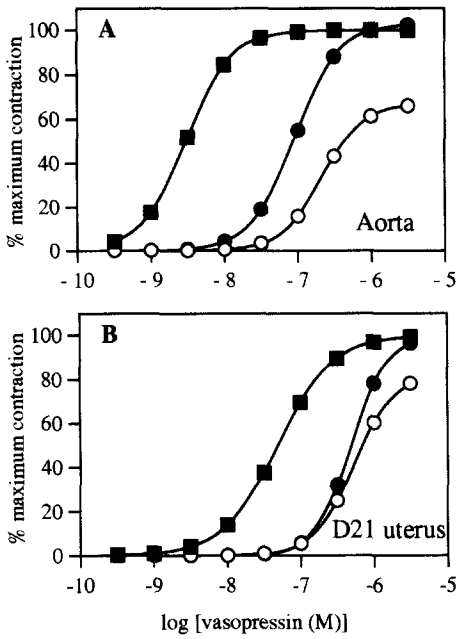

Fig. 1. Vasopressin contractile response as percent of maximum response for rat thoracic aorta (A) and uterus (B) isolated from day 21 pregnant rats. Responses obtained in the absence (■) or presence of d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin (●) (100 nM) or d(CH$_2$)$_5$-[Tyr(Me)^2]vasopressin (○, 1 nM in A, 100 nM in B). Curves are theoretical curves calculated from the average computed parameters listed in Table 1.

Table 1
Contractile responses to vasopressin of rat aorta rings and of circularly oriented strips taken from day 21 pregnant rat uterus

Agonist		Vasopressin	
Antagonist	Control	d(CH$_2$)$_5$[Tyr(Me)^2]AVP	d(CH$_2$)$_5$[Tyr(Me)^2]OVT
Concentration	None	1 nM	100 nM
Aorta			
E_{max} (mg/2 min)	3673 ± 40 [a]	2466 ± 16 [b]	3769 ± 23 [a]
−log EC_{50}	8.52 ± 0.07	6.67 ± 0.01	7.04 ± 0.01
n	(6)	(6)	(6)
Concentration	None	100 nM	100 nM
D21 uterus			
E_{max} (mg/2 min)	1126 ± 254 [c]	938 ± 179 [d]	1130 ± 389 [d]
−log EC_{50}	7.28 ± 0.15	6.27 ± 0.27	6.31 ± 0.23
n	(3)	(6)	(6)

Responses were studied in the absence and presence of d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin or d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin. Data are means (±S.E.M.) of the average value for 4 strips; *n*, number of animals. a versus b, $P < 0.05$. c versus d, $P > 0.05$.

compared using Student's unpaired t-test after log transformation of values to ensure homogeneity of variances. Significance was set at $P < 0.05$.

3. Results

3.1. Contractile response to vasopressin in aorta and uterine strips

Fig. 1 and Table 1 compare the contractile response curves to vasopressin in aorta and in D21-pregnant uterus in the absence and presence of two related antagonists. When added alone, each antagonist displayed no effect on aortic or uterine contraction up to the 1 μM concentration. In aorta, d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin very potently antagonized the effect of vasopressin. At the low 1 nM concentration it depressed the amplitude of E_{max} by 1/3 and also provoked a 70-fold shift of the curve to the right (apparent pK_B = 10.84). Conversely, d(CH$_2$)$_5$[Tyr(Me)^2]-

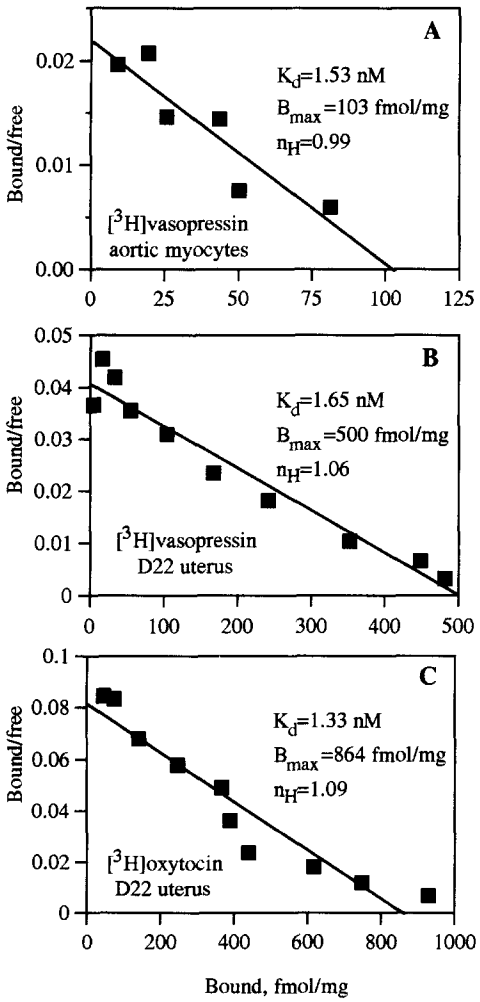

Fig. 2. Equilibrium binding of [^{3}H]vasopressin (A and B) and [^{3}H]oxytocin (C) to membranes prepared from cultured aortic myocytes (A) and from day 22 pregnant uterus (B and C). The values illustrated in each graph are from one experiment.

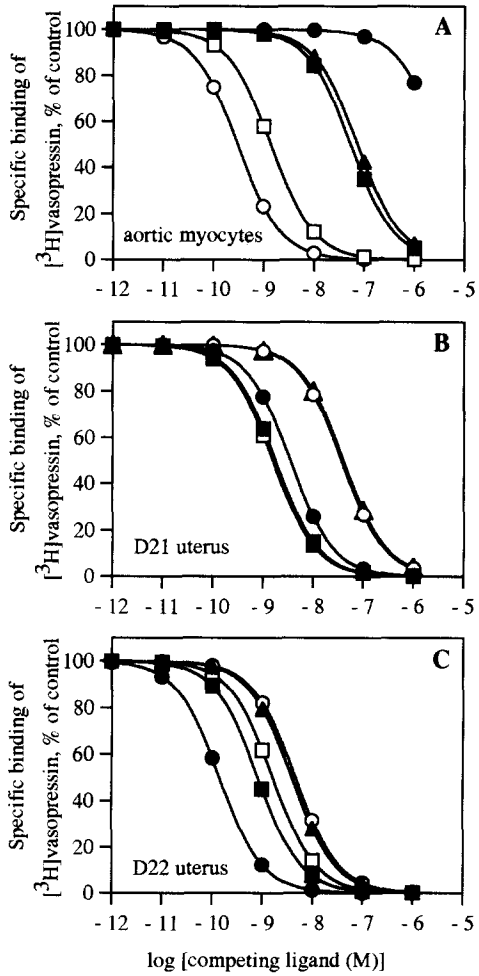

Fig. 3. Families of self- and cross-displacement curves between [^{3}H]vasopressin and vasopressin (□), oxytocin (■), [Thr^4,Gly^7]oxytocin (●), d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (○) and d(CH$_2$)$_5$[Tyr(Me)^2]-ornithine vasotocin (▲). Membranes prepared from cultured aortic myocytes (A), day 21 pregnant uterus (B) and day 22 pregnant uterus (C). Membranes incubated in the presence of a fixed (0.5 nM) concentration of tracer and increasing concentrations of unlabelled peptides (1 pM to 1 μM). Ordinate: percentage of specific binding; abscissa: log molar concentration of unlabelled ligands. Curves redrawn from curves generated by the Ebda-Ligand program.

ornithine vasotocin had no effect on E_{max} and was much less potent than d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin at inhibiting the vasopressin response. At 1 nM d(CH$_2$)$_5$[Tyr-(Me)^2]ornithine vasotocin there was no right-shift of the vasopressin curve (data not shown) while 100 nM provoked a 30-fold right-shift of the vasopressin curve (pK_B = 8.47).

In the non-parturient uterus on D21 of gestation, d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin and d(CH$_2$)$_5$[Tyr(Me)^2]-ornithine vasotocin at 1 nM concentration had no statistically significant effect on E_{max} and they did not inhibit vasopressin response (data not shown). At 100 nM, they still had no effect on E_{max} but they inhibited vasopressin-induced contractile responses in a similar manner (pK_B = 7.97).

Thus, in aorta and D21-pregnant uterus the potency of the two antagonists was very different. Moreover, it is noteworthy that vasopressin was more potent and induced a very large contraction in aorta which was 3-fold greater than in D21 uterus, for strips of equivalent wet weight.

3.2. Binding studies

[^{3}H]Vasopressin bound with high affinity to a single population of sites in membrane preparations from aortic myocytes and parturient D22 uterus, as determined by Scatchard analysis of equilibrium saturation binding (K_d = 2.63 ± 1.66 nM and K_d = 1.72 ± 0.39 nM, respectively, n = 3) (Fig. 2A,B). In D22 uterus, the affinity for [^{3}H]oxytocin was in the same range (K_d = 1.21 ± 0.34 nM, n = 3) but the maximum binding density was considerably higher for [^{3}H]oxytocin (B_{max} = 850 ± 175 fmol/mg protein) than for [^{3}H]vasopressin (B_{max} = 500 ± 88 fmol/mg protein) at the same stage of gestation (Fig. 2C).

Fig. 3A-C illustrates in membranes from aortic myocytes and from D21 and D22 uterus the result of simultaneous analysis of self- and cross-inhibition curves for five unlabelled peptides competing with [^{3}H]vasopressin binding. In all cases, the model that best fitted the raw data involved only one independent class of sites. In aorta membranes, [^{3}H]vasopressin was hardly displaced by [Thr^4,Gly^7]oxytocin (K_i > 1000 nM) whereas vasopressin and d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin had the same high affinity (K_i = 0.2–0.7 nM). Oxytocin and d(CH$_2$)$_5$-[Tyr(Me)^2]ornithine vasotocin (K_i = 27–37 nM) had intermediate affinity situated between these two extreme limits. In contrast, in D21-uterus membranes, vasopressin and oxytocin (K_i = 1 nM) showed no distinguishing selectivity for inhibiting the binding of [^{3}H]vasopressin; d(CH$_2$)$_5$-[Tyr(Me)^2]ornithine vasotocin (K_i = 20 nM) had the same affinity as in aorta, d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (K_i = 18 nM) had less affinity than in aorta, and [Thr^4,Gly^7]oxytocin (K_i = 2 nM) exhibited a dramatic increase in affinity compared to that in aorta. Finally, in D22 uterus membranes, the affinities for oxytocin and vasopressin were almost the same as those measured in D21 uterus (K_i = 0.4–0.8 nM). However, the affinities for [Thr^4,Gly^7]oxytocin, d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin and d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (K_i = 0.3–2 nM) were increased as compared to those in D21 uterus (Table 2).

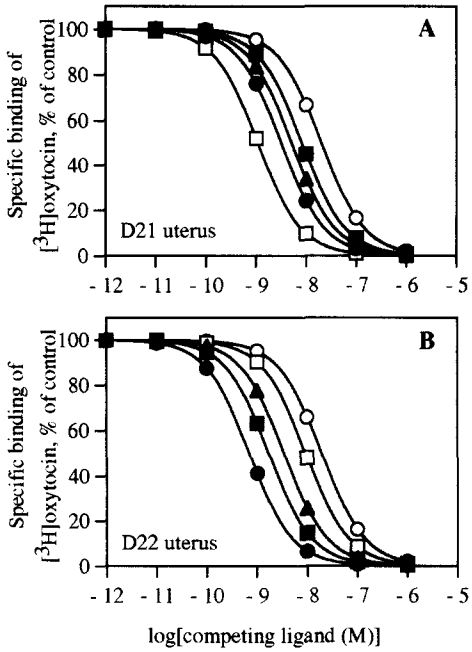

Fig. 4. Families of self- and cross-displacement curves between [^{3}H]oxytocin and vasopressin (□), oxytocin (■), [Thr^4,Gly^7]oxytocin (●), d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (○) and d(CH$_2$)$_5$[Tyr(Me)^2]-ornithine vasotocin (▲). Membranes prepared from day 21 pregnant uterus (A) and day 22 pregnant uterus (B). Membranes incubated in the presence of a fixed (0.5 nM) concentration of tracer and increasing concentrations of unlabelled peptides (1 pM to 1 μM). Ordinate: percentage of specific binding; abscissa: log molar concentration of unlabelled ligands. Curves redrawn from curves generated by the Ebda-Ligand program.

Table 2
Competition of oxytocin, vasopressin and their analogs for [^{3}H]vasopressin binding sites in membranes prepared from cultured aortic myocytes and from day 21 and day 22 pregnant rat uterus

Competing ligand	K_i (nM)		
	[^{3}H]Vasopressin		
	Aorta	D21 uterus	D22 uterus
Oxytocin	27 ± 3.22 [a]	0.88 ± 0.20 [b]	0.41 ± 0.11 [c]
[Thr^4,Gly^7]oxytocin	> 1000 [a]	1.74 ± 0.39 [b]	0.27 ± 0.02 [c]
d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin	37 ± 16.09 [a]	20 ± 4.40 [a]	1.94 ± 0.81 [b]
Vasopressin	0.69 ± 0.57 [a]	0.78 ± 0.19 [a]	0.81 ± 0.68 [a]
d(CH$_2$)5[Tyr(Me)^2]vasopressin	0.15 ± 0.05 [a]	18.30 ± 4.99 [b]	2.30 ± 0.63 [c]

Competition binding experiments were carried out with 0.5 nM labelled ligand as described in Material and methods. K_i values for competing ligands were generated by the Ebda-Ligand program for each series of experiments. Values are the means (± S.E.M.) of three independent determinations analyzed in duplicate with the Ebda-Ligand program. [a,b,c] Different superscripts in the same row indicate significant differences between values.

Table 3
Competition of oxytocin, vasopressin and their analogs for [^{3}H]oxytocin binding sites in membranes prepared from day 21 and from day 22 pregnant rat uterus

Competing ligand	K_i (nM)	
	[^{3}H]Oxytocin	
	D21 uterus	D22 uterus
Oxytocin	4.09 ± 1.08 [a]	0.86 ± 0.50 [b]
[Thr^4,Gly^7]oxytocin	1.58 ± 0.14 [a]	0.34 ± 0.18 [b]
d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin	2.55 ± 0.47 [a]	1.73 ± 0.57 [a]
Vasopressin	0.54 ± 0.16 [a]	4.63 ± 0.89 [b]
d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin	9.95 ± 2.01 [a]	9.72 ± 5.23 [a]

Competition binding experiments were carried out with 0.5 nM labelled ligand as described in Material and methods. K_i values for competing ligands were generated by the Ebda-Ligand program for each series of experiments. Values are the means (± S.E.M.) of three independent determinations analyzed in duplicate with the Ebda-Ligand program. [a,b]Different superscripts in the same row indicate significant differences between values.

Fig. 4A,B shows the result of simultaneous analysis of self- and cross-inhibition curves for the same previous five unlabelled peptides used to inhibit [^{3}H]oxytocin binding in D21- and D22-uterus membranes. The model that best fitted the raw data, as for [^{3}H]vasopressin competition, involved only one independent class of sites. Clearly, on D21, the affinitiy of vasopressin (K_i = 0.5 nM) was higher than that of the four other peptides (K_i = 2–10 nM). Inversely, on D22, the affinities of vasopressin (K_i = 5 nM) and d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin (K_i = 10 nM) were significantly lower than those of the other peptides (K_i = 0.3–2 nM). d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin had the same affinity on D22 as on D21 uterus (Table 3).

The relative potencies of the different ligands, measured on D21 or D22 uterus membrane, for [^{3}H]oxytocin and [^{3}H]vasopressin are compared in Fig. 5. The abscissa displays the log of K_i values of competing ligands for [^{3}H]oxytocin binding and the ordinate indicates the log of K_i values of competing ligands for [^{3}H]vasopressin binding. Both hormones and analogs had a potency ratio of less than 10, however the distribution of potency ratios was different for D21 uterus membranes compared to that for D22 uterus membranes.

4. Discussion

Several lines of evidence have raised the question of a possible heterogeneity of oxytocin receptors in the uterus. Saturation (Pliska et al., 1986) and competition studies with tritiated oxytocin, vasopressin or lysine-vasopressin (Guillon et al., 1987; Maggi et al., 1988, 1990; Tence et al., 1990) have revealed the presence of at least two categories of sites linking oxytocin and vasopressin (or lysine-vasopressin) that have been named oxytocin and V_1 receptors, respectively. Also, the pharmacological response to oxytocin dramatically increases at parturition and this appears to occur mainly in the circular layer of the rat myometrium (Fuchs et al., 1983; Crankshaw, 1987; Garfield and Beier, 1989; El Alj et al., 1993). The principal questions addressed in this paper were whether oxytocin and vasopressin bind to separate oxytocin and vasopressin receptors in the uterus and whether uterine vasopressin receptors are the same as the vascular V_{1A} receptor subtype. Because the pharmacological receptor subtype definition is closely dependent on the animal species (Hall et al., 1993), we directly compared our rat myometrium data with our findings in aorta of the same species.

We first examined whether the vasopressin-induced

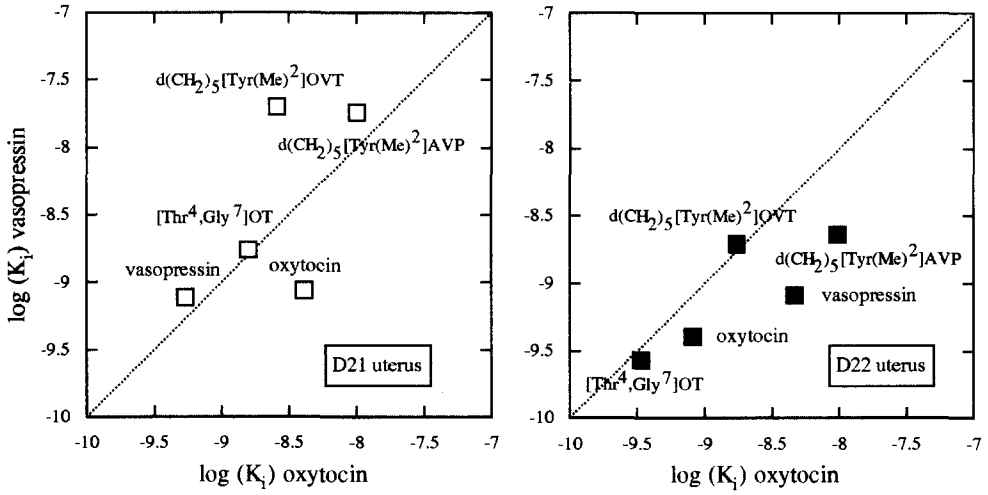

Fig. 5. Graphical representation of the selectivity of several peptides for the binding of [^{3}H]oxytocin and [^{3}H]vasopressin to membranes prepared from day 21 pregnant uterus (□) and from day 22 pregnant uterus (■). Abscissa, log (K_i) for [^{3}H]oxytocin. Ordinate, log (K_i) for [^{3}H]vasopressin. The K_i values were calculated by the Ebda-Ligand program and are given in Tables 2 and 3. The doted line indicates equipotency of competing ligand for inhibiting [^{3}H]oxytocin and [^{3}H]vasopressin binding. [Thr^4,Gly^7]OT, [Thr^4,Gly^7]oxytocin; d(CH$_2$)$_5$[Tyr(Me)^2]AVP, d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin; d(CH$_2$)$_5$[Tyr(Me)^2]OVT, d(CH$_2$)$_5$[Tyr(Me)^2]ornithine vasotocin.

contractile response of circular muscle was mediated in the D21-pregnant uterus by vasopressin receptors that are the same as those present in aortic vascular smooth muscle. Comparison of the displacement of the vasopressin response in aorta and in D21 uterus by vasopressin antagonists $d(CH_2)_5$[Tyr(Me)^2]vasopressin and $d(CH_2)_5$[Tyr(Me)^2]ornithine vasotocin demonstrated that vasopressin receptors were different in the two tissues. $d(CH_2)_5$[Tyr(Me)^2]ornithine vasotocin had no clearly different selectivity for aortic or uterine vasopressin receptors, but $d(CH_2)_5$[Tyr(Me)^2]vasopressin at a low concentration of 1 nM was very efficient at antagonizing vasopressin in aorta whereas it had no effect at the same concentration in the D21 uterus. It is noticeable that under our experimental conditions $d(CH_2)_5$[Tyr(Me)^2]vasopressin showed an apparent non-competitive effect in aorta, as demonstrated by its effect on E_{max} decrease. It is difficult to explain this effect on E_{max} in aorta because our binding study demonstrated a competitive inhibition of [^{3}H]vasopressin binding by $d(CH_2)_5$[Tyr(Me)^2]vasopressin on membranes from cultured aortic myocytes (see below). Because we did not scrape off the aorta endothelium we may hypothesize that the decrease in E_{max} was related to the influence of secondarily activated relaxing factors that were released from the endothelium following vasopressin + $d(CH_2)_5$[Tyr(Me)^2]vasopressin exposure. It is possible that these were not entirely controlled in our experiments although we strove to eliminate them by preincubating the aorta with indomethacin and N^{ω}-nitro-L-arginine. It is known for example that the release of endothelium-dependent relaxing factors can be activated in aorta by analogs of vasopressin (Yamada et al., 1993) and we did observe a relaxation in intact aorta stimulated by vasopressin in the absence of indomethacin and N^{ω}-nitro-L-arginine (unpublished data). In any case, from our results it is unlikely that receptors inducing a tonic response to vasopressin in the D21 uterus are of the V_{1A}-subtype. Only a high concentration of $d(CH_2)_5$[Tyr(Me)^2]vasopressin was effective in inhibiting the vasopressin response in the D21 pregnant uterus. This effect was hence different from that seen in aorta. This is in agreement with the results of Chan et al. (1990) who compared in vivo the antioxytocic : antivasopressor ratios of several peptidic oxytocin receptor antagonists and found that each peptide exhibited a different potency ratio ranging from high to low values (0.9–0.1). These authors concluded from the lack of unity in the potency ratios among these oxytocin receptor antagonists that the myometrial receptors, although having somewhat vasopressor-like profiles, were not the same as the V_{1A} receptors found in vascular smooth muscle tissue.

Binding experiments were carried out on the whole uterus and aortic myocytes. We confirmed the presence of saturable high affinity binding sites for [^{3}H]vasopressin and [^{3}H]oxytocin in these tissues. We found that Scatchard plots were essentially linear for both ligands in aortic myocytes and pregnant uterus. K_d values were in close agreement with those reported by others in the same tissues (Fuchs et al., 1983; Chan et al., 1990; Pettibone et al., 1992; Serradeil-Le Gal et al., 1995). However, saturation curves failed to distinguish between vasopressin and oxytocin binding sites because the two ligands have the same affinity.

Competition curves of [^{3}H]vasopressin with unlabelled oxytocin, vasopressin and three of their synthetic analogs were further compared in membranes from aortic myocytes in culture and from the pregnant uterus. Essentially, the potency of $d(CH_2)_5$[Tyr(Me)^2]vasopressin and [Thr^4,Gly^7]oxytocin at antagonizing [^{3}H]vasopressin binding confirmed the differences between vascular smooth muscle and uterine vasopressin receptors. $d(CH_2)_5$-[Tyr(Me)^2]vasopressin had higher affinity for vascular smooth muscle cell sites than for D21 or D22 uterine sites. Interestingly, the apparent pK_B (10.84) calculated for $d(CH_2)_5$[Tyr(Me)^2]vasopressin in aorta was close to the pK_i (9.82) value found in aortic myocytes. Likewise, the peptide exhibited lower pK_B (7.97) and pK_i (7.74) values when measured in D21 uterus. This confirmed our findings observed in functional studies. These results are also in accordance with the 1 : 100 effective antagonistic antioxytocic : antivasopressor dose-ratio reported in vivo for $d(CH_2)_5$[Tyr(Me)^2]vasopressin by Manning and Sawyer (1984). On the contrary, we found that $d(CH_2)_5$[Tyr(Me)^2]ornithine vasotocin had no selectivity for vascular and uterine vasopressin receptors. This is a direct confirmation of the equivalent potencies of the latter peptide for its in vivo antioxytocic and antivasopressor properties as reported by Manning and Sawyer (1989). The potent oxytocic peptide [Thr^4,Gly^7]oxytocin (Lowbridge et al., 1977) was the next compound that best discriminated between vasopressin receptors in aortic myocyte membranes and uterus membranes. In aortic myocytes, the K_i for [Thr^4,Gly^7]oxytocin was similar to that found in other tissues expressing the V_{1A} receptor subtype (Kiraly et al., 1986). In D22 uterus, the K_i for this analog was the same as that found for the oxytocin receptor subtype by Elands et al. (1988). The intermediate K_i value that we found for [Thr^4,Gly^7]oxytocin in D21 uterus could therefore tentatively be explained by the binding of this analog to the V_{1A} receptor and oxytocin receptor subtypes expressed at the same time in this tissue. However, if this was true, the slope of the competition curve should have been very low, such as that found in hippocampal membranes for OH[Thr^4,Gly^7]oxytocin, a [Thr^4,Gly^7]oxytocin analog, by Audigier and Barberis (1985). Because this was not the case in our current study, we have to conclude that the vasopressin receptor we identified in our contractility and binding studies on the uterus was different from the classical V_{1A} receptor of vascular tissue.

It was more difficult to distinguish specific pharmacological differences between oxytocin and vasopressin receptors in the uterus. As reported by Chan et al. (1990), we found on D22 of gestation that the difference in B_{max}

values between [^{3}H]oxytocin and [^{3}H]vasopressin binding was high, 850 fmol/mg versus 500 fmol/mg respectively, suggesting that there were two different binding sites. Chan et al. (1990) also reported that the affinity and maximum binding value for [^{3}H]vasopressin in the rat uterus was the same on D21 and D22 of gestation suggesting that what happens on D22 is probably the appearance of a new population of oxytocin receptors. From this, one may expect a good level of vasopressin receptors in the rat uterus on D21 and a good level of oxytocin receptors on D22 of gestation. Therefore we checked whether the peptides we used had a different selectivity for the binding of [^{3}H]oxytocin and [^{3}H]vasopressin to uterine membranes on either D21 or D22 of gestation. In fact, the five peptides showed different potency ratios for [^{3}H]oxytocin and [^{3}H]vasopressin suggesting that different receptors were expressed in the uterus at these two stages of gestation. However these differences were limited, precluding the possibility of unambiguously distinguishing two categories of receptors. On D22, the high affinity of [Thr^4,Gly^7]oxytocin and oxytocin for both [^{3}H]oxytocin and [^{3}H]vasopressin binding sites was consistent with the notion that the uterus expresses essentially oxytocin receptors at this stage of gestation. Nevertheless, it has been shown that oxytocin receptors can be regulated by guanine-nucleotide-binding proteins (Marc et al., 1988), and a possibility could be that the limited differences we observed between D21 uterus and D22 uterus for the affinities of oxytocin and analogs might represent in fact low and high affinity states of one single oxytocin receptor. It has been shown on cultured astroglial cells that oxytocin receptors, like a number of other receptors, can have two interconvertible states of high and low affinity, in which the high affinity state is stabilized by the interaction with a guanine nucleotide regulatory protein to form a ligand-G-protein ternary complex (Di Scala-Guenot and Strosser, 1992). Nervertheless, two points argue against this interpretation in the uterus. On the one hand, Crankshaw et al. (1990) reported that the affinities of the two oxytocin sites they identified in the non-pregnant rat uterus by the affinity spectrum method were not influenced by the presence of 10 μM GTPγS in the incubation medium, and we have previously verified that competition curves constructed with data for [Thr^4,Gly^7]oxytocin in D21 uterus [^{3}H]oxytocin labelled membranes did not differ in the presence and absence of 10 μM GTPγS (unpublished data). On the other hand, the affinity of antagonists is believed, in general, not to be affected in the same way as agonists by the state of the receptor (Kent et al., 1980), although in our study we found the same trend for the changing affinity of agonists and antagonists in the uterus between D21 and D22 of gestation. An alternative hypothesis could be that oxytocin and its analogs might have bound in the D21 uterus to another receptor belonging to the family of neurohypophysial hormone receptors, namely the V_{1B} receptor. It was recently revealed by RNA blot analysis that V_{1B} receptor mRNA is expressed not only in the majority of pituitary corticotrophs but also in various peripheral tissues including the uterus (Lolait et al., 1995). However, the affinity of d(CH$_2$)$_5$[Tyr(Me)^2]vasopressin for the V_{1B} receptor is approximately 200 nM while we found it was at least 10-fold less in uterine membranes, rendering the presence of such a receptor in our preparations unlikely.

In conclusion, unlike in humans, the vasopressin receptor that we identified in our contractility and binding study on the rat uterus is different from the classical V_{1A} receptor of vascular tissue. We also found that the binding sites for [^{3}H]vasopressin and [^{3}H]oxytocin on day 21 uterus membranes do not resemble the classical oxytocin receptor as described in the literature, suggesting that on day 21 vasopressin and oxytocin bind in the uterus to a receptor that might be different from those currently characterized. Likewise, another study group recently identified on a pharmacological basis separate uterotonic oxytocin receptors and decidual prostaglandin-releasing oxytocin receptors in pregnant rats, favoring the idea of the presence of distinct oxytocin receptor subtypes in the uterus (Chan et al., 1993; Chen et al., 1994). Additional studies will be necessary to clarify the precise molecular nature of these receptors. The recent cloning of the rat oxytocin receptor (Rozen et al., 1995; Adan et al., 1995) and the related rat V_{1A} (Morel et al., 1992) and V_{1B} receptors (Saito et al., 1995; Lolait et al., 1995) allied to the detection in the uterus of reverse-transcription polymerase chain reaction products obtained with degenerate primers that could amplify their cDNAs, will be helpful in this respect, as has been reported for the vascular-type vasopressin receptor (Hirasawa et al., 1994).

Acknowledgements

We are indebted to M. Manning for providing the synthetic analogs of oxytocin and vasopressin, and to A.J. Strickland for English corrections. Part of this work was done while A.A., M-S.C. and G.G. were at Unité Inserm 361, Paris, France. These authors are grateful to the staff members of this research unit, in particular to Dr F. Cavaillé, for the help they received to initiate the experiments.

References

Adan, R.A.H., F.W. Van Leeuven, M.A.F. Sonnemans, M. Brouns, G. Hoffman, J.G. Verbalis and J.P.H. Burbach, 1995, Rat oxytocin receptor in brain, pituitary, mammary gland, and uterus: partial sequence and immunocytochemical localization, Endocrinology 136, 4022.

Akerlund, M., P. Stromberg, A. Hauksonn, L. Franch Andersen, J. Lyndrup, J. Trojnar and P. Melin, 1987, Inhibition of uterine contrac-

tions of premature labor with an oxytocin analogue. Results from a pilot study, Br. J. Obstet. Gynaecol. 94, 1040.

Alexandrova, M. and M.S. Soloff, 1980a, Oxytocin receptors and parturition. I. Control of oxytocin concentration in the rat myometrium at term, Endocrinology 106, 730.

Alexandrova, M. and M.S. Soloff, 1980b, Oxytocin receptors and parturition. II. Concentration of receptor for oxytocin and oestrogen in the gravid and non gravid uterus at term, Endocrinology 106, 736.

Arunlakshana, O. and H.O. Schild, 1959, Some qualitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Audigier, S. and C. Barberis, 1985, Pharmacological characterization of two specific binding sites for neurohypophysial hormones in hippocampal synaptic plasma membranes of the rat, Eur. Mol. Biol. Organ. J. 4, 1407.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Caldeyro-Barcia, R. and J.A. Sereno, 1959, The response of the human uterus to oxytocin throughout pregnancy, in: Oxytocin, eds. R. Caldeyro-Barcia and H. Heller (Pergamon Press, London) p. 177.

Chan, W.Y., L. Cao, S.P. Hill and V.J. Hruby, 1990, Oxytocin- and vasopressin-binding sites in the rat uterus: competition binding and inhibitory pA_2 studies with oxytocin and oxytocin antagonists, Endocrinology 126, 2095.

Chan, W.Y., D.L. Chen and M. Manning, 1993, Oxytocin receptor subtypes in the pregnant rat myometrium and decidua: pharmacological differentiations, Endocrinology 132, 1381.

Chen, D.L., W.Y. Chan and M. Manning, 1994, Agonist and antagonist specificities of decidual prostaglandin-releasing oxytocin receptors and moymetrial uterotonic oxytocin receptors in pregnant rats, J. Reprod. Fert. 102, 337.

Crankshaw, D., V. Gaspar and V. Pliska, 1990, Multiple [^{3}H]-oxytocin binding sites in rat myometrial plasma membranes, J. Receptor Res. 10, 269.

Crankshaw, D., 1987, The sensitivity of the longitudinal and circular muscle layers of the rat's myometrium to oxytocin in vitro during pregnancy, Can. J. Physiol. Pharmacol. 65, 773.

Di Scala-Guenot, D., and M.T. Strosser, 1992, Oxytocin receptors on cultured astroglial cells. II. Regulation by a guanine-nucleotide-binding protein and effect on Mg^{2+}, Biochem. J. 284, 499.

El Alj, A., N. Winer, H. Lallaoui, R. Delansorne, F. Ferre and G. Germain, 1993, Progesterone and mifepristone modify principally the responses of circular myometrium to oxytocin in preparturient rats: comparison with responses to acetylcholine and to calcium, J. Pharmacol. Exp. Ther. 265, 1205.

Elands, J., C. Barberis and S. Jard, 1988, [^{3}H]-[Thr^4,Gly^7]OT: a highly selective ligand for central and peripheral OT receptors, Am. J. Physiol. 254, E31.

Fuchs, A.R., S. Periyasamy, M. Alexandrova and M.S. Soloff, 1983, Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids, Endocrinology 113, 742.

Garfield, R.E. and S. Beier, 1989, Increased myometrial responsiveness to oxytocin during term and preterm labor, Am. J. Obstet. Gynecol. 161, 454.

Guillon, G., M.N. Balestre, J.M. Roberts and S.P. Bottari, 1987, Oxytocin and vasopressin: distinct receptors in myometrium, J. Clin. Endocrinol. Metab. 64, 1129.

Hall, J.M., M.P. Caulfield, S.P. Watson and S. Guard, 1993, Receptor subtypes or species homologues: relevance to drug discovery, Trends Pharmacol. Sci. 14, 376.

Hirasawa, A., K. Shibata, K. Kotosai and G. Tsujimoto, 1994, Cloning, functional expression and tissue distribution of human cDNA for the vascular-type vasopressin receptor, Biochem. Biophys. Res. Commun. 203, 72.

Ito, Y., O. Kozawa, H. Tokuda, A. Suzuki, Y. Watanabe, J. Kotoyori and Y. Oiso, 1994, Glucocorticoid inhibits cAMP production induced by vasoactive agents in aortic smooth muscle cells, Atherosclerosis 110, 69.

Ivanisevic, M., O. Behrens, H. Helmer, K. Demarest and A.R. Fuchs, 1989, Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists, Am. J. Obstet. Gynecol. 161, 1637.

Kent, R., S., A. Delean and R.J. Lefkowitz, 1980, A quantitative analysis of β-adrenergic receptor interaction: Resolution of high and low affinity states of the receptor by computer modeling of ligand binding data, Mol. Pharmacol. 17, 14.

Kiraly, M., S. Audigier, E. Tribollet, C. Barberis, D. Dolivo and J.J. Dreyfuss, 1986, Biochemical and electrophysiological evidence of functional vasopressin receptors in the rat superior cervical ganglion, Proc. Natl. Acad. Sci. USA 83, 5335.

Lolait, S.J., A.M. O'Carrol, L.C. Mahan, C.C. Felder, D.C. Button, W.S. Young III, E. Mezey and M.J. Brownstein, 1995, Extrapituitary expression of the rat V1b vasopressin receptor gene, Proc. Natl. Acad. Sci. USA 92, 6783.

Lowbrige, J.M., M. Manning, J. Haldar and W.H. Sawyer, 1977, Synthesis and some pharmacologiccal properties of [4-threonine, 7-glycine]oxytocin, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 4-threonine, 7-glycine]oxytocin (hydroxy[Thr4,Gly7]oxytocin) and [7-glycine]oxytocin, peptides with high oxytocic-antidiuretic selectivity, J. Med. Chem. 20, 120.

Maggi, M., A.D. Genazzani, S. Giannini, C. Torrisi, E. Baldi, M. Di Tomasso, P.J. Munson, D. Rodbard and M. Serio, 1988, Vasopressin and oxytocin receptors in vagina, myometrium, and oviduct of rabbits, Endocrinology 122, 2970.

Maggi, M., P. Del Carlo, G. Fantoni, S. Giannini, C. Torrisi, D. Casparis, G. Massi and M. Serio, 1990, Human myometrium during pregnancy contains and responds to V_1 vasopressin receptors as well as oxytocin receptors, J. Clin. Endocrinol. Metab. 70, 1142.

Manning, M. and W.H. Sawyer, 1984, Design and uses of selective agonistic and antagonistic analogs of the neuropeptides oxytocin and vasopressin, Trends Neurosci. 7, 6.

Manning, M. and W.H. Sawyer, 1989, Discovery, development, and some uses of vasopressin and oxytocin antagonists, J. Lab. Clin. Med. 114, 617.

Marc, S., D. Leiber and S. Harbon, 1988, Fluoroaluminates mimic muscarinic- and oxytocin-receptor mediated generation of inositol phosphates and contraction in the intact guinea-pig myometrium, Biochem. J. 255, 705.

Morel, A., A.M. O'carroll, M.J. Brownstein and S.J. Lolait, 1992, Molecular cloning and expression of rat V_{1a} arginine vasopressin receptors, Nature 356, 523.

Munson, P.J. and D. Rodbard, 1980, LIGAND: A versatile computerized approach for the characterization of ligand binding systems, Anal. Biochem. 107, 220.

Pettibone, D.J., M.T. Kishel, C.J. Woyden, B.V. Clineschmidt, M.G. Bock, R.M. Freidinger, D.F. Veber and P.D. Williams, 1992, Radioligand binding studies reveal marked species differences in the vasopressin V_1 receptor of rat, rhesus and human tissues, Life Sci. 50, 1953.

Pliska, V., J. Heiniger, A. Müller-Lhotsky, P. Pliska and B. Ekberg, 1986, Binding of oxytocin to uterine cells in vitro: occurrence of several binding site populations and reidentification of oxytocin receptors, J. Biol. Chem. 261, 16984.

Rozen, F., C. Russo, D. Banville and H.H. Zing, 1995, Structure, characterization, and expression of the rat oxytocin receptor gene, Proc. Natl. Acad. Sci. USA 92, 200.

Saito, M., T. Sugimoto, A. Tahara and H. Kawashima, 1995, Molecular cloning and characterization of rat V1B vasopressin receptor: evidence for its expression in extra-pituitary tissues, Biochem. Biophys. Res. Commun. 212, 751.

Serradeil-Le Gal, C., J.M. Hebert, C. Delisee, P. Schaeffer, D. Raufaste,

C. Garcia, F. Dol, E. Marty, J.P. Maffrand and G. Le Fur, 1995, Effect of SR-49059, a vasopressin V_{1a} antagonist, on human vascular smooth muscle cells, Am. J. Physiol. 268, H404.

Tence, M., G. Guillon, S. Bottari and S. Jard, 1990, Labelling of vasopressin and oxytocin receptors from the human uterus, Eur. J. Pharmacol. 191, 427.

Yamada, K., M. Nakayama, H. Nakano, N. Mimura and S. Yoshida, 1993, Endothelium-dependent vasorelaxation evoked by desmopressin and involvement of nitric oxide in rat aorta, Am. J. Physiol. 264, E203.